TEXT SEARCHABLE DOCUMENT

DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** Bardac 22C50 (Carboquat) **PC Code No.:** 069208

2. **TEST MATERIAL:** Carboquat **Purity:** 49.85%
   Batch No.: 5628-137

3. **CITATION**

   Authors: Debbie Desjardins, B.S.
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   Henry O. Krueger, Ph.D

   Title: A 96-Hour Toxicity Test of Bardac 22C50 with the Freshwater Alga (*Anabaena flos-aquae)*

   Study Completion Date: May 19, 2004

   Laboratory: Wildlife International, Ltd.
   8598 Commerce Drive
   Easton, Maryland 21601

   Sponsor: Lonza Inc.
   17-17 Route 208
   Fair Lawn, New Jersey 07410

   Laboratory Report ID: 289A-158

   DP Barcode: D305528

   MRID No.: 462958-01

4. **REVIEWED BY:** Kathryn V. Montague, Biologist US EPA/AD/RASSB

   Signature: **Date:**

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader US EPA/AD/RASSB

   Signature: **Date:**

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: *Anabaena flos-aquae*
   Definitive Test Duration: 96 Hours

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Type of Concentrations: Day 0 Measured (5.4, 9.4, 18, 40, and 75 Φg a.i./L)

7. CONCLUSIONS

Results Synopsis (Cell Density)

96-hr:

EC_{50}: 58 Φg a.i./L 95% C.I.: 49 and 69 Φg a.i./L
NOAEC: 40 Φg a.i./L

72-hr:

EC_{50}: 64 Φg a.i./L 95% C.I.: 60 and 67 Φg a.i./L

48-hr:

EC_{50}: 54 Φg a.i./L 95% C.I.: 48 and 61 Φg a.i./L

24-hr:

EC_{50}: 67 Φg a.i./L 95% C.I.: 65 and 70 Φg a.i./L

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable (Core)

B. Rationale: No significant deviations from Guideline requirements.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.5400 (EPA 712-C-96-164):

$ The pH exceeded the required pH of 7.5 ∀ 0.1 at exposure termination. The study report states that this is typical for tests conducted with *Anabaena flos-aquae*, due to increases in algal densities.

$ The standard deviation was not calculated or plotted and the goodness-of-fit was not determined.

$ The following information was not reported:
- Age of stock culture
- How much photosynthetically active radiation test chambers received
- Results and methods for range-finding test
- If the laboratory runs positive controls with zinc chloride as a reference chemical periodically to ensure that the test algae are responding to a known chemical in an expected manner
- The maximum labeled rate of the test substance
- If the analytical method was validated

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species $ *Selenastrum capricornatum (Raphidocelis subcapitata)* $ *Skeletonema costatum* $ *Anabaena flos-aquae* $ *Navicula pelliculosa*	$ *Anabaena flos-aquae*
Initial Number of Cells $ 10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* $ 77,000 cells/mL (*Skeletonema*)	$ 10,000 cells/mL (p.14)
Stock Culture $ 3 to 7 days old	$ Not reported
Nutrients	

Guideline Criteria	Reported Information
$ Standard formula (ASTM E1218-20)	$ Nutrient solutions were prepared by adding reagent-grade chemicals to purified Wildlife International, Ltd. well water. (p.11)
$ pH 7.5 ∀ 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ∀ 0.1 (*Skeletonema*)	$ pH = 7.4 (p.11)
$ Freshly prepared	$ Yes

B. Test System

Guideline Criteria	Reported Information
Solvent $ Upper limit - 0.5 mL/L	$ No solvent used
Temperature $ 24E ∀ 2EC (*Selenastrum, Navicula, Anabaena*) $ 20E ∀ 2EC (*Skeletonema*) $ Recorded hourly	$ Test chambers were held in an environmental chamber in which the temperature was set to 24 ∀ 2EC. The temperature of a container of water set adjacent to the test chambers in the environmental chamber was recorded twice daily. Water temperatures recorded using a hand held thermometer ranged from 22.8 EC to 23.9 EC . The minimum and maximum temperatures measured continuously throughout the study were 23 and 24 EC. (p.16) $ Air temperature (which is more likely to fluctuate than solution temperature) was measured continuously using a Fulscope Recorder and a min/max thermometer.
Light Intensity $ 4.3 K lx (∀ 10%) (*Selenastrum, Skeletonema, Navicula*) $ 2.2 K lx (∀ 10%) (*Anabaena*) $ Photosynthetically active radiation approx. 66.5 ∀ 10% ΦEin/m^2/sec	$ 2170 to 2280 lux (p.17) $ Not Reported

Guideline Criteria	Reported Information
Photoperiod $ 14-hr light/10-hr dark (*Skeletonema*) $ Continuous (*Selenastrum, Navicula, Anabaena*)	$ Continuous
pH $ 7.5 ∀ 0.1 (*Selenastrum, Navicula, Anabaema*) $ 8.1 ∀ 0.1 (*Skeletonema*) $ Measured at beginning and end of test	$ The pH ranged from 7.1 at test initiation to 9.2 at exposure termination. The Study Author reported that the pH was believed to have increased relative to increases in algal densities, typical for tests conducted with *Anabaena flos-aquae*. (p.16) $ Yes (p.12)
Oscillation Rates $ 100 cycles/min (*Selenastrum*) $ 60 cycles/min (*Skeletonema*)	$ The test flasks were shaken continuously at 100 rpm. (p.12)
Test Containers $ 125-500 mL Erlenmeyer flasks $ Cleaned/sterilized (solvent and acid) and conditioned $ Test solution volume # 50% of flask volume	$ 250-mL Erlenmyer flasks (p.12) $ Sterilized and pretreated for 24 hours with Bardac 22C50 solution of each respective treatment (p.12) $ Each flask contained 100 mL of test solution or control medium. (p.12)
Dilution Water $ Sufficient quality (e.g., ASTM Type I) $ Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	$ Purified Wildlife International, Inc. well water (p.11)

C. Test Design

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Range-Finding Test $ Water solubility and physical-chemical properties of test chemical determined? $ Validated analytical method developed? $ Expose algae to widely spaced (e.g., log interval) chemical concentration series $ Lowest value should be at detection limit $ Upper value, for water soluble compounds, should be at saturation concentration $ Minimum of 3 replicates $ Algae should be exposed for 96 hours $ If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, a definitive test may not be necessary $ If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	$ An exploratory range-finding test was conducted to determine the nominal test concentrations; however, the methods and results of the range-finding test were not reported. (p.10) $ An analytical method was developed by Wildlife International, Ltd. No information was provided on the validation of the method. (p.13)
Dose Range $ 1.5X -2X progression	$ 2X progression
Doses $ 5 or more concentrations of test substance in a geometric series $ > 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	$ Geometric series of 5 concentrations. Nominal concentrations were 4.7, 9.4, 19, 38, and 75 Φg a.i./L. Day 0 measured concentrations were 5.4, 9.4, 18, 40, and 75 Φg a.i./L. $ 94% inhibition of growth at 75 Φg a.i./L (p.17)
Controls $ Negative and/or solvent each test $ Positive - zinc chloride (periodically)	$ Negative control $ Not Reported
Replicates Per Dose $ 3 or more (4 or more for *Navicula*)	$ 3 replicates (p.10)
Duration of Test $ 96-hr	$ 96-hr

Guideline Criteria	Reported Information
Growth $ Logarithmic growth (controls) by 96-hr or repeat test (increase by a factor of 16) $ 1.5 x 10^6 cells/mL (*Skeletonema*) $ 3.5 x 10^6 cells/mL (*Selenastrum*)	$ Growth in controls increased by a factor of 119 with mean cell density increasing to 1,193,333 cells/mL in the negative control by 96-hr. (p.24)
Daily Observations?	Yes (p1.4)
Method of Observations $ Direct - microscopic cell count of at least 400 cells/flask $ Indirect - spectrophotometry, electronic cell counter, dry weight, etc.; calibrated by microscopic count $ Qualitative and descriptive	$ The cell counts were performed using a hemacytometer and microscope. (p.15). The minimum quantifiable cell density was 1,000 cells/mL. (p.15)
Cell Separation $ Syringe, ultrasonic bath, or blender; limited sonification (*Anabaena*) $ Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula)*	$ Sample solutions were drawn in and out of a syringe to shorten the length of the cell chains (p.15) $ Test flasks were shaken continuously at 100 rpm. (p. 12).
Algistatic and algicidal effects differentiated?	Yes, based on the growth observed in the recovery phase, the effect on algal growth at the highest concentration was found to be algistatic. (p.17)
Maximum Labeled Rate	Not Reported

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in the report?	Yes
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	Yes, parent stock of *Anabaena flos-aquae* was obtained from the University of Toronto Culture Collection and had been maintained in culture medium at Wildlife International, Ltd., Easton, Maryland. (p.11)
Growth in controls reported?	Yes
Description of test system and test design included?	Yes
Initial and final chemical concentrations and pH measured?	Yes (p.12 and 13)
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	Yes (p.23)
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	Yes
Raw data included?	Yes (p.45)
Methods and data records reported?	Yes
Statistical Analysis $ Mean and standard deviation calculated and plotted? $ Goodness-of-fit determined?	 $ Standard deviation not calculated or plotted $ No

Dose Response at 96-hr

Day 0 Measured Test Concentration (Φg a.i./L)	Mean Cell Density (cells/mL)	% Inhibition (reduction in growth compared with control)	pH	
			0-hr	96-hr
Negative Control	1,193,333	--	7.2	9.2
4.7	1,168,333	2.1	7.2	8.8
9.4	1,165,000	2.4	7.1	8.8
19	1,196,667	-0.28	7.1	8.7
38	1,165,000	2.4	7.1	8.6
75	75,333	94	7.2	8.3

Statistical Results

Statistical Method: Non-linear regression was used to calculate EC_{50} values and their corresponding 95% confidence intervals for cell density (EC_{50}) for each 24-hour exposure period, when possible. The 96-hour NOAEC was calculated by evaluating the data for normality and homogeneity of variance (p=0.05) using the Shapiro-Wilk=s and Levene=s tests, respectively. This was followed by comparison of the treatment groups to the negative control using Dunnett=s test (p=0.05).

The Day 0 measured test concentrations were used to calculate the EC_{50} and NOAEC values. The Day 0 measured test concentrations ranged from 96 to 115% of nominal concentrations and the Day 4 measured test concentrations ranged from 34 to 90% of nominal concentrations.

96-hr (Cell Density):

EC_{50}: 58 Φg a.i./L **95% C.I.:** 49 and 69 Φg a.i./L
NOAEC: 40 Φg a.i./L

72-hr (Cell Density):

EC_{50}: 64 Φg a.i./L 95% C.I.: 60 and 67 Φg a.i./L

48-hr (Cell Density):

EC_{50}: 54 Φg a.i./L 95% C.I.: 48 and 61 Φg a.i./L

24-hr (Cell Density):

EC_{50}: 67 Φg a.i./L 95% C.I.: 65 and 70 Φg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

96-hour cell density statistics were verified using ANOVA with Dunnett's Test (TOXSTAT). The results show that the 75 Φg a.i./L cell density was significantly lower than the control cell density, which is in agreement with the reported results. Linear regression was used to verify the EC50 results, which were also in general agreement with the reported results.

14. REVIEWER=S COMMENTS:

- $ Guideline deviations are noted in Section 9.
- $ There was one amendment noted in the Study Protocol: (1) Based on the results of the range-finding study, the test concentrations were changed from 20, 30, 44, 67, and 100 Φg a.i./L to 4.7, 9.4, 19, 38, and 75 Φg a.i./L to facilitate the determination of a NOAEC.

Sign-off Date : 11/04/05
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